

090306-0000

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[SEQ ID NO: 7]

[SEQ ID NO: 8]

[SEQ ID NO: 9]

[SEQ ID NO: 10]

[SEQ ID NO: 11]

[SEQ ID NO: 12]

[SEQ ID NO: 13]

Table 2. Sequence of PCR primers for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG
TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG
TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG
TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG
WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG
TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG
TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR
TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1f: 5'-ACT GCC TCC ACC ACC GCT GCC ACC TCC GCC AGA TCC
TCC GCC GCC TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC CAG
GGT G-3' [SEQ ID NO: 21]

0060363-063900

Vk4b: 5'-GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GAA ACG ACA CTC ACG CAG
TCT C-3' [SEQ ID NO: 41]

3'-primers (forward primers):

Vk1f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT TTC CAC CTT GGT
CC-3' [SEQ ID NO: 42]

Vk2f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT CTC CAS CTT GGT
CC-3' [SEQ ID NO: 43]

Vk3f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT GAT ATC CAC TTT GGT
CC-3' [SEQ ID NO: 44]

Vk4f: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TTC TTT AAT CTC CAG TCG TGT
CC-3' [SEQ ID NO: 45]

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5'-TCGA GGC GGT GGT GGA TCA GGC GGC GGA GGA TCT GGC GGA
GGT GGC AGC GGT GGT GGA GGC AGT GCG CGC TTA ATT AA-3'
[SEQ ID NO: 46]

5'-TCG ATT AAT TAA GCG CGC ACT GCC TCC ACC ACC GCT GCC
ACC TCC GCC AGA TCC TCC GCC GCC TGA TCC ACC ACC GCC-3'
[SEQ ID NO: 47]

Table 4. Sequence of PCR primers that include loxP sites for amplifying heavy- and light-chain genes of human antibody.

(B= C/G/T; D= A/G/T; K= G/T; M= A/C; R= A/G; S= C/G; W= A/T; and Y= C/T)

a) Heavy-chain VH

5'-primers (back primers):

VH1b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG CAG GAG
TCS G-3' [SEQ ID NO: 14]

VH2b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTA CAG CTG CAG CAG
TCA-3' [SEQ ID NO: 15]

VH3b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTA CAG CAG
TGG G-3' [SEQ ID NO: 16]

VH4b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC GAG GTG CAG CTG KTG GAG
WCY-3' [SEQ ID NO: 17]

VH5b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTC CAG CTK GTR CAG
TCT GG-3' [SEQ ID NO: 18]

VH6b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG RTC ACC TTG AAG GAG
TCT G-3' [SEQ ID NO: 19]

VH7b: 5'-ACC CCA CCA AAC CCA AAA AAA GAG ATC TGT ATG GCT
TAC CCA TAC GAT GTT CCA GAT TAC CAG GTG CAG CTG GTG SAR
TCT GG-3' [SEQ ID NO: 20]

3'-primers (forward primers):

VH1'f: 5'-ACT GCC TCC ACC TGA TAA CTT CGT ATA GCA TAT ATT
ATA CGA AGT TAT TGA TCC ACC ACC GCC TGA GGA GAC RGT GAC
CAG GGT G-3' [SEQ ID NO: 48]

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Vk4'b: 5'-GGC GGT GGT GGA TCA ATA ACT TCG TAT AAT ATA TGC
TAT ACG AAG TTA TCA GGT GGA GGC AGT GAA ACG ACA CTC ACG
CAG TCT C-3' [SEQ ID NO: 68]

3'-primers (forward primers):

Vk1'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT
TTC CAC CTT GGT CC-3' [SEQ ID NO: 69]

Vk2'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT
CTC CAS CTT GGT CC-3' [SEQ ID NO: 70]

Vk3'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT GAT
ATC CAC TTT GGT CC-3' [SEQ ID NO: 71]

Vk4'f: 5'-CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT TTT AAT
CTC CAG TCG TGT CC-3' [SEQ ID NO: 72]

3'-primers (forward primers) for 2nd PCR of Vλ and Vk:

Vλ/Vkf: 5'-GAG ATG GTG CAC GAT GCA CAG TTG AAG TGA ACT TGC
GGG GTT TTT CAG TAT CTA CGA TAA CTT CGT ATA ATG TAT GCT-
3' [SEQ ID NO: 73]

006290" 6960960

SEQUENCE LISTING

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Hua, Shaobing

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ANTIBODY REPERTOIRE IN YEAST

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<210> 11
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<210> 13

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<213> Artificial Sequence

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85

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